CLAIMS

1. Biologically active isolated Tat protein, fragments thereof and/or mutants and/or Tat DNA for use as a vaccine, said Tat, at picomolar to nanomolar concentrations being capable of: (i) entering and localizing in the nuclei of activated endothelial cells or dendritic cells; and/or (ii) activating the proliferation, migration and invasion of Kaposi's sarcoma (KS) cells and cytokine-activated endothelial cells protein.
2. Biologically active isolated Tat protein, fragments thereof and/or mutants and/or DNA Tat according to claim 1 further capable of: (iii) activating virus replication when added to infected cells as measured a) by the rescue of Tat-defective proviruses in HLM-1 cells after the addition of exogenous protein; and/or b) by the transactivation of HIV-1 gene expression in cells transfected with a HIV-1 promoter-reporter plasmid.
3. Biologically active isolated Tat protein, fragments thereof and/or mutants and/or Tat DNA according to claim 2 further capable of: (iv) inducing in mice the development of KS-like lesions in the presence of angiogenic factors or inflammatory cytokines.
4. Biologically active isolated Tat protein, fragments thereof and/or mutants and/or Tat DNA according to claims 1-3 at amounts ranging between 10 ng/ml or less to 1 μg/ml.
5. Biologically active isolated Tat protein, fragments thereof and/or mutants and/or Tat DNA according to claims 1-4 for use in the prophylactic and/or therapeutic treatment of AIDS, tumors, syndromes and symptoms associated with HIV infection.
6. Protein or peptide or DNA vaccine, prophylactic and/or therapeutic, against AIDS, tumors, syndromes and symptoms associated with the HIV infection, comprising biologially active Tat and/or its mutants and/or portion of the protein or peptides or a DNA as defined in claims 1-4.
7. Vaccine according to claim 6 in which Tat has the following nucleotide sequence (Seq.1) :

5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTC

AGCCTAAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCATTG
CCAAGTTTGTTTCATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAAGAA
GCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAGTTT
CTCTATCAAAGCAGCCCACCTCCCAATCCCGAGGGGACCCGACAGGCCC
GAAGGAATAG 3'

and any other Tat variant of any HIV type and subtype.

8. Vaccine according to claim 6 in which Tat has the following amino acid sequence :

NH2-EPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKAISY
GRKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDPTGPKE-COOH

and any other Tat variant of any HIV type and subtype.

9. Vaccine according to claim 6 in which mutants are selected among the ones having the following nucleotide sequences or part of them:

Nucleotide sequence of cys22 mutant (Seq.2)

5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT
CAGCCTAAAACTGCGGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCATT
GCCAAGTTTGTTTCATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAAG
AAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAG
TTTCTCTATCAAAGCAGCCCACCTCCCAATCCCGAGGGGACCCGACAGG
CCCGAAGGAATAG 3'

Nucleotide sequence of lys41 (Seq.3)

5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT
CAGCCTAAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCAT
TGCCAAGTTTGTTTCATAACAAACGCCTTAGGCATCTCCTATGGCAGGAA
GAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAA
GTTTCTCTATCAAAGCAGCCCACCTCCCAATCCCGAGGGGACCCGACAG
GCCCGAAGGAATAG 3'

Nucleotide sequence of RGDΔ mutant (Seq.4)

5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT
CAGCCTAAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCAT
TGCCAAGTTTGTTTCATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAA

GAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAA
GTTTCTCTATCAAAGCAGCCCACCTCCCAATCCCCGACAGGCCCGAAGG
AATAG 3'

Nucleotide sequence of lys41-RGDΔ mutant (Seq.5)

5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT
CAGCCTAAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCAT
TGCCAAGTTTGTTTCATAACAAACGCCTTAGGCATCTCCTATGGCAGGAA
GAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAA
GTTTCTCTATCAAAGCAGCCCACCTCCCAATCCCCGACAGGCCCGAAGG
AATAG 3'

10. Vaccine according to claim 6 in which mutants are selected among the ones having the following amino acid sequence or part of them:

Amino acid sequence of cys22 mutant

NH2-MEPVDPRLEPWKHPGSQPKTAGTNCYCKKCCFHCQVCFITKA
LGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDPTGPKE-COOH

Amino acid sequence of lys41

NH2-MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITTAL
GISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDPTGPKE-COOH

Amino acid sequence of RGDΔ mutant

NH2-MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKAL
GISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSPTGPKE-COOH

Amino acid sequence of lys41-RGDΔ mutant

NH2-MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITTAL
GISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSPTGPKE-COOH

11. Vaccine according to claim 6 in which the Tat portions are selected among the peptide sequences

Pep. 1. MEPVDPRLEPWKHPGSQPKT

Pep. 2. ACTNCYCKKCCFHCQVCFIT

Pep. 3. QVCFITKALGISYGRK

Pep. 4. SYGRKKRRQRRRPPQ

Pep. 5. RPPQGSQTHQVSLSKQ

Pep. 6. HQVSLSKQPTSQSRGD

Pep. 7. PTSQSRGDPTGPKE

12. Vaccine according to claims 6-11 comprising proteins or peptides conjugated with the T-helper universal epitope of Tetanus toxoid or any T-helper peptides.

13. Vaccine according to claims 6-12 in combination with recombinant proteins or peptides of HIV Nef, Rev or Gag or part of them.

14. Vaccine according to claim 6 comprising fusion proteins Tat (wild type or its mutants)/Nef, Tat (wild type or its mutants)/Rev, Tat (wild type or its mutants)/Gag or part of them.

15. Vaccine according to claim 6-14 in combination with recombinant immuno-modulant cytokines or other molecules, or part of them, augmenting antiviral immune response.

16. Vaccine according to claim 15 in which cytokines are IL-12 and/or IL-15 or IFNα or IFNβ.

17. Vaccine according to claim 6 comprising fusion proteins Tat(wild type or its mutants)/immuno-modulant cytokines, Tat (wild type or its mutants)/IL-12, Tat (wild type or its mutants)/IL-15, Tat (wild type or its mutants)/other molecules, or part of them, augmenting the antiviral immune response.

18. DNA vaccine according to claims 6, 7, 9 comprising DNA encoding for Tat wild-type or its mutants or part of them, inserted in expression vectors.

19. DNA vaccine according to claims 6, 7, 9 in combination with an expression vector including HIV rev, nef and gag genes, or part of them.

20. DNA vaccine according to claims 18 or 19 in which the vector is a plasmid co-expressing tat (wild-type or its mutants)/rev, tat (wild-type or its mutants)/nef, tat (wild-type or its mutants)/gag or part of them.

21. DNA vaccine according to claims 6, 7, 9 in combination with DNA molecules inserted in expression vectors encoding for immuno-modulant cytokines or other immuno-modulant molecules, or part of them, augmenting the antiviral immune response.

22. DNA vaccine according to claim 21 in which the cytokine is IL-12 and/or IL-15.

23. DNA vaccine according to claims 21 or 22 in which the vector is a plasmid co-

expressing tat (wild-type or its mutants)/IL-12, tat (wild-type or its mutants)/IL-15, tat (wild-type or its mutants)/other molecules, or part of them, able to augment the antiviral immune response.

24.Vaccine according to claims 18-23 in which the vector is pCV0.

25.Vaccine according to the previous claims including autologous dendritic cells treated and/or untreated according to the previous claims.

26.Vaccine according to the previous claims including adjuvants able to augment the antiviral immune response.

27.Vaccine according to claim 26 in which the adjuvant is selected among Alum, ISCOM, RIBI and related mixtures.

28.Vaccine according to previous claims comprising systems for delivery.

29.Vaccine according to claim 28 in which the systems for delivery are selected among nanoparticles, herpes vectors, red cells, bacteria and combinations thereof.

30.Vaccine according to claim 29 in which bacteria are selected among *Streptococcus gordonii* and *Lactobacillus*.

31.Vaccine according to claims 29 and 30 in which bacteria are modified to express viral antigens.

32.Vaccine according to the previous claims for the immunization of peripheral blood cells from infected individuals, expanded by co-stimulation with magnetic beads coated with anti-CD3 and anti-CD28 antibodies.

33.Therapeutic vaccine according to the previous claims, combined with inhibitors of viral replication.

34.Vaccine according to claims 6-33 in which the active principle is delivered to the mucosa.

35. Vaccine according to claim 34, in which the active principle is administered nasally, orally, vaginally and/or rectally.

36.Vaccine according to claims 6-33 in which the active principle is administered through systemic or local route.

37.Vaccine according to claim 36 in which the active principle is administered through intramuscular, subcute or intradermal route.

38.Vaccine according to claim 37 in which the active principle is administered intradermally at 1-6 μg amounts, without adjuvants.

39.Vaccine according to claims 34-38 in which the active principle is carried in a biologically acceptable fluid.

40.Vaccine according to claims 34-39 further comprising pharmaceutically acceptable carriers and eccipients to maximize the principle activity.

41.Vaccine according to claims 34-40 comprising as active principle Tat according to claim 1 at preventive and/or therapeutic amounts.

42. Biologically active Tat nucleotide sequence (Seq. 1):

5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT
CAGCCTAAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCAT
TGCCAAGTTTGTTTCATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAA
GAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAA
GTTTCTCTATCAAAGCAGCCCACCTCCCAATCCCGAGGGGACCCGACAG
GCCCGAAGGAATAG 3'

43.Biologically active Tat amino acid sequence

NH2-EPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKAISY
GRKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDPTGPKE-COOH

44.Biologically active Tat mutant protein having nucleotide sequence selected among:

Nucleotide sequence of the cys22 mutant (Seq.2)

5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT
CAGCCTAAAACTGCGGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCATT
GCCAAGTTTGTTTCATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAAG
AAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAG
TTTCTCTATCAAAGCAGCCCACCTCCCAATCCCGAGGGGACCCGACAGG
CCCGAAGGAATAG 3'

Nucleotide sequence of the lys41 mutant (Seq.3)

5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT
CAGCCTAAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCAT
TGCCAAGTTTGTTTCATAACAAACGCCTTAGGCATCTCCTATGGCAGGAA

GAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAA
GTTTCTCTATCAAAGCAGCCCACCTCCCAATCCCGAGGGGACCCGACAG
GCCCGAAGGAATAG 3'

Nucleotide sequence of the RGDΔ mutant (Seq.4)

5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT
CAGCCTAAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCAT
TGCCAAGTTTGTTTCATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAA
GAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAA
GTTTCTCTATCAAAGCAGCCCACCTCCCAATCCCCGACAGGCCCGAAGG
AATAG 3'

Nucleotide sequence of the lys41-RGDΔ mutant (Seq.5)

5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT
CAGCCTAAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCAT
TGCCAAGTTTGTTTCATAACAAACGCCTTAGGCATCTCCTATGGCAGGAA
GAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAA
GTTTCTCTATCAAAGCAGCCCACCTCCCAATCCCCGACAGGCCCGAAGG
AATAG 3'

45.Biologically active Tat mutants amino acid sequence selected among:

Amino acid sequence of cys22 mutant

NH2-MEPVDPRLEPWKHPGSQPKTAGTNCYCKKCCFHCQVCFITKA
LGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDPTGPKE-COOH

Amino acid sequence of lys41 mutant

NH2-MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITTAL
GISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDPTGPKE-COOH

Amino acid sequence of RGDΔ mutant

NH2-MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKAL
GISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSPTGPKE-COOH

Amino acid sequence of lys41-RGDΔ mutant

NH2-MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITTAL
GISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSPTGPKE-COOH

46.Biologically active Tat mutants with peptide sequence selected among :

Pep. 1. MEPVDPRLEPWKHPGSQPKT

Pep. 2. ACTNCYCKKCCFHCQVCFIT

Pep. 3. QVCFITKALGISYGRK

Pep. 4. SYGRKKRRQRRRPPQ

Pep. 5. RPPQGSQTHQVSLSKQ

Pep. 6. HQVSLSKQPTSQSRGD

Pep. 7. PTSQSRGDPTGPKE

47.Expression vector comprising a DNA sequence selected among the ones listed in claims 42 and 44 or parts thereof.

48.Expression vector pCV0 including a DNA sequence selected among the ones listed in claims 42-44 or parts thereof.

49.Expression vector pCV0 according to claim 48, comprising a DNA sequence codifying for a gene selected among tat, rev, nef, gag, IL-12, IL-15 and combinations thereof.

50.Transformed cells including the vector according to claims 47-49.

51.Dendritic cells treated with Tat protein or its peptides or mutants according to claims 42-46 or combinations with Rev, Nef, and Gag proteins and/or cytokines.

52.Dendritic cells according to claim 51 transduced with an expression vector comprising tat gene.

53.Process for producing biologically active Tat protein or its mutants or its recombinant forms or parts of them comprising to cultivate the cells according to claim 50 and to isolate and purify the thus obtained protein or parts of it.

54.Process for preparing the pCV0 vector according to claim 49, in which the corresponding cDNA is amplified by PCR technique using primers selected among:

Seq. P1. Primer forward Rev: 5'ATGGCAGGAAGAAGC3'

Seq. P2. Primer reverse Rev: 5'CTATTCTTTAGTTCC3'

Seq. P3. Primer forward Nef: 5'ATGGGTGGCAAGTGG3'

Seq. P4. Primer reverse Nef: 5'TCAGCAGTCCTTGTA3'

Seq. P5. Primer forward Gag: 5'ATGGGTGCGAGAGCG3'

Seq. P6. Primer reverse Gag: 5'TTATTGTGACGAGGG3'

Seq. P7. Primer forward IL-12: 5'ATGTGGCCCCCTGGG3'

Seq. P8. Primer reverse IL-12: 5'TTAGGAAGCATTCAG3'

Seq. P9. Primer forward IL-15: 5'ATGAGAATTTCGAAA3'

Seq. P10. Primer reverse IL-15: 5'TCAAGAAGTGTTGAT3'

Seq. P11.Primer forward Tat: 5'ATGGAGCCAGTAGAT3'

Seq. P12. Primer reverse Tat: 5'CTATTCCTTCGGGCC3'

Seq. P13. Primer forward Tat/Rev: 5'GGCCCGAAGGAAATGGCA GGAAGAAGC3'

Seq. P14. Primer forward Tat/Nef: 5' GGCCCGAAGGAAATGGGT GGCAAGTGG3'

Seq. P15. Primer forward Tat/Gag: 5' GGCCCGAAGGAAATGGGT GCGAGAGCG3'

Seq. P16. Primer forward Tat/IL-12: 5' GGCCCGAAGGAAATGTGGC CCCCTGGG3'

Seq. P17. Primer forward Tat/IL-15: 5' GGCCCGAAGGAAATGAGAAT TTCGAAA3'

55.Primer selected among:

Seq. P1. Primer forward Rev: 5'ATGGCAGGAAGAAGC3'

Seq. P2. Primer reverse Rev: 5'CTATTCTTTAGTTCC3'

Seq. P3. Primer forward Nef: 5'ATGGGTGGCAAGTGG3'

Seq. P4. Primer reverse Nef: 5'TCAGCAGTCCTTGTA3'

Seq. P5. Primer forward Gag: 5'ATGGGTGCGAGAGCG3'

Seq. P6. Primer reverse Gag: 5'TTATTGTGACGAGGG3'

Seq. P7. Primer forward IL-12: 5'ATGTGGCCCCCTGGG3'

Seq. P8. Primer reverse IL-12: 5'TTAGGAAGCATTCAG3'

Seq. P9. Primer forward IL-15: 5'ATGAGAATTTCGAAA3'

Seq. P10. Primer reverse IL-15: 5'TCAAGAAGTGTTGAT3'

Seq. P11.Primer forward Tat: 5'ATGGAGCCAGTAGAT3'

Seq. P12. Primer reverse Tat: 5'CTATTCCTTCGGGCC3'

Seq. P13. Primer forward Tat/Rev: 5'GGCCCGAAGGAAATGGCA

GGAAGAAGC3'

Seq. P14. Primer forward Tat/Nef: 5' GGCCCGAAGGAAATGGGT GGCAAGTGG3'

Seq. P15. Primer forward Tat/Gag: 5' GGCCCGAAGGAAATGGGT GCGAGAGCG3'

Seq. P16. Primer forward Tat/IL-12: 5' GGCCCGAAGGAAATGTGGC CCCCTGGG3'

Seq. P17. Primer forward Tat/IL-15: 5' GGCCCGAAGGAAATGAGAAT TTCGAAA3'.

56.Process for preparing a vaccine according to claims 6-41, wherein Tat is in its non oxidated form.

57.Process for preparing a vaccine according to claims 6-41, wherein Tat, in its lyophilized form, is re-suspended in a biologically acceptable fluid for administration.

58.Use of Tat protein wild-type in its active form and/or its mutants and/or parts related to the protein or peptides or the DNA encoding for these proteins or parts of them or peptides to make a protein or peptide or DNA vaccine, preventive and/or therapeutic, against AIDS, tumors, the syndromes and symptoms associated to HIV infection.

59.Use of Alum, ISCOM, RIBI and other adjuvants, alone or in combination, to make a vaccine according to claim 6.

60.Use of paramagnetic beads coated with monoclonal antibodies anti-CD3 and anti-CD28 to make a vaccine according to claim 6.

61.Therapeutic method for treating AIDS, tumors, syndromes and symptoms associated with HIV infection characterized in that preventive or therapeutic amounts of biologically active Tat according to claims 1-5 are administered.

HIV-1 Tat, or derivatives thereof, alone or in combination, for prophylactic and therapeutic vaccination against AIDS, tumors and related syndromes

Abstract

The present invention refers to Tat as the active principle for a prophylactic and/or therapeutic vaccine against HIV infection, the progression towards AIDS and the development of tumors and other syndromes and symptoms in subjects infected by HIV. Tat is in biologically active form either as recombinant protein or peptide or as DNA. More particularly, the invention refers to a vaccine based on HIV-1 Tat as immunogen, inoculated as DNA and/or recombinant protein or as peptides, alone or in combination with other genes or viral gene products (Nef, Rev, Gag) or parts thereof, or in combination with various immuno modulant cytokines (IL-12, IL-15) or with the gene coding for an immuno modulant cytokine or part thereof. Tat, Nef, Rev, Gag and the immuno modulant cytokines are administrated both as a mixture of recombinant proteins, peptides or fusion proteins (Tat/Nef, Tat/Rev, Tat/Gag, Tat/IL-12, Tat/IL-15) or as plasmid DNA.